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The Impact of an Inert Gas Atmosphere on the Kinetics of Changes in the Physical and Chemical Properties of Carrot Lyophilisate

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Abstract:
The purpose of this study was to analyse the impact of blanching, the atmosphere of nitrogen and storage time on carotenoids content, colour and sorption properties of freeze-dried carrot. The material was stored for up to 16 weeks, in the dark, at room temperature. The colour was measured on the surface and in cross sections. As the storage time increased, there was a continual decrease in the carotenoids content in the carrot packed in atmospheric air. In the carrots packed in the atmosphere of nitrogen, no changes in carotenoid content were observed. The colour parameters correlated with the changes in the carotenoids content. The colour parameters changed on the surface and in cross sections in the same manner. During storage, a significant decrease in sorption properties of freeze-dried carrots was noted after 2 weeks of storage.

Keywords: carrot, freeze-drying, nitrogen, storage, carotenoid content, colour, sorption properties
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1 Introduction

Lyophilisation is a method of drying which generates a higher quality product when compared to products obtained after using other methods of drying: convective, infrared, vacuum, spray drying, microwave or combinations of these methods. Lyophilisation has a positive impact on such qualities as: colour, smell, nutritional value, porosity and rehydration ability [1–3].

Carrots are one of the most important dried vegetables. They are a valuable resource due to their availability and high antioxidant content, especially β-carotene. For instance, β-carotene has been found to reduce the risk of cancer [4, 5] and to increase immune response [6]. Carrots are recommended to people facing the possibility of Vitamin A deficiency [7].

Bioactive compounds in dried foods may be subject to degradation when stored. Chen et al. [8] noted that the carotenoids in carrots contain a highly unsaturated molecule, so pigments are subjected to isomerization, which causes colour loss and oxidation which results in a decrease in the nutritional value of carrots when stored.

Pesek and Warthesen [9] found that the β-carotene content decreased as storage temperature and time increased. Yen et al. [10] noted that after 30 days of storage the carotenoid content in freeze-dried carrots fell more than two-fold, while in air-dried carrots, it was lower than in freeze-dried carrots, but it did not change during storage. This was caused by the changes in the porosity of the material during the drying process. Fruit and vegetable lyophilisates have a highly porous structure [11, 12] reaching 80–90 %. Porosity is a desired feature of dried foods, *inter alia*, due to its rheological properties and rehydration ability. However, due to high porosity the ingredients of dried matter are in good contact with the air that fills the structure, which may result in the oxidation of some bioactive components. In material dried in the warm air, oxidation takes place during the process itself; however, as a result of shrinkage during storage oxidation is reduced. One of the methods of reducing the loss of nutrients in freeze-dried foods may be the treatment of carrots with ascorbic acid (0.1 %) in a glucose solution (1 %) before drying [10]. However, the application of the proposed treatments may lead to the leaching of minerals from the raw material. It is also an additional technological operation, which results in increased water consumption and additional production of wastewater. Moreover, it provides protection only on the surface, not in the whole volume.

Therefore, increasing the stability of carotenoids during storage is an important issue. It is necessary to plan procedures which would receive the products with a high nutritional concentration, unchanged during storage.

D. Nowak is the corresponding author.

2 Materials and methods

The research material was the Karotka carrot obtained in a local store Piotr i Paweł, Warsaw, Poland. Fresh carrots were cut into 1 cm thick slices. Blanched and unblanched carrot was freeze-dried. The blanching was carried out in water at 95 °C for 1 min.

Before lyophilisation the material (ca 400 g) was frozen for 2 h at −40 °C in a shock freezer with forced air circulation (IRINOX S.P, Italy, model MF 25.1 PLUS).

Lyophilisation was conducted in the laboratory freeze-drier, model Alfa 1–4 (CHRIST, Germany; Ice condenser capacity 4 kg; Ice Condenser temperature – 55 °C.) with the following parameters: shelf temperature: 10 °C, pressure: 63 Pa, and drying time: 20 h.

Depending on the experiment variant, on completion of the process, the freeze-dryer chamber was filled with atmospheric air or nitrogen (supplied from the attached cylinder).

The material was packed in a three-layer foil (PE/AL/PP) of high air and vapour barrier properties. The material was packed in the air or in atmosphere of nitrogen. It was stored at an ambient temperature of 22–23 °C for 2, 4, 8 and 16 weeks.

There were nine variants of the experiment which are described in Table 1.

Table 1: The variants of the experiment.

Sample mark	Pre-treatment	Underpressure reduced with	Gas in the packaging
BL_AA	blanching	air	air
BL_AN	blanching	air	nitrogen
BL_NN	blanching	nitrogen	nitrogen
NB_AA	none	air	air
NB_AN	None	air	nitrogen
NB_NN	None	nitrogen	nitrogen

2.1 Determination of water content

The water content in the material was determined with the use of reduced-pressure drying in accordance with the PN-ISO 1026:2000 P norm. A VO500 Memmert vacuum dryer was used at 70 °C, and the pressure of 10 mbar, for 24 h. The samples were weighed on analytical scales with the accuracy of 0.0001 g. The experiment was carried out in three iterations.

2.2 Determination of the total carotenoid content

The carotenoid content was determined with the use of the spectrophotometric method (PN-EN 12136:2000), by measuring the absorbance of the carotenoid solution in a 1 cm thick glass cell, at a wave length of 450 nm, with a Helios γ Thermo Electron spectrophotometer UVG (Thermo Spectronic, Great Britain). The carotenoids were extracted with the acetone (20 mL, thrice) and petroleum ether (25 mL). The experiment was conducted in two parallel tests with two iterations for each trial.

2.3 Determination of the parameters of colour

The colour was determined by a Minolta CR-A70 measurement instrument (Minolta Co., Ltd, Osaka, Japan), in the CIE Lab system, by measuring trichromatic components a*, b*, L*. Each test was conducted in five iterations. The total colour difference (ΔE) was calculated using the formula (1):

$$\Delta E = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2} \quad (1)$$

The lyophilisate, immediately after drying was the reference material.

2.4 Determination of the kinetics of sorption

Research material (one carrot slice) was placed on a scale suspended under the balance, above the saturated solution of sodium chloride (water activity = 0.75). Vibra analytical scales were used with an accuracy of 0.0001 g. The weight measurements were taken every 2 min for 24 h with the use of the RTS for Windows software. Based on the measurement results, the relative increase in water content during sorption was determined using the formula (2):

$$\frac{\Delta u_\tau}{u_0} = \frac{u_\tau - u_0}{u_0} = \frac{m_\tau - m_0}{m_0 \cdot ss_o} \tag{2}$$

where,

u_τ – water content in dried foods (g water/g d.m.) after the τ time period,
u_o – water content in dried foods (g water/g d.m.) at the beginning of sorption,
m_τ – the weight of one carrot slice after τ sorption time (g),
m_o – the initial weight of one carrot slice (g),
ss_o – the content of dry matter, a fraction.

A multifactor analysis of the variations was carried out with the use of the Statistica 13.3 software. To analyse whether the applied pre-treatment method the way of removing negative pressure from the freeze-dryer chamber and the storage conditions impact the specific properties of dried foods, the Tukey's range test and one-dimensional results, with the significance level of $\alpha = 0.05$, were used.

3 Results and discussion

3.1 Carotenoids content

The fresh carrots used in the research contained 100.15 ± 1.19 mg/100 g d.m. carotenoids. Nowak and Matyjek [13] obtained the value of 136 mg/100 g d.m. carotenoids in fresh carrots. Lee et al. [14] studied carrots containing 85 ± 12 mg/100 g d.m. These differences resulted from the variations of the biological material used [15].

The carrot blanched immediately after drying contained an average of 103.52 ± 6.55 mg/100 g d.m., while the unblanched ones – 96.50 ± 2.50 mg/100 g d.m. The difference in carotenoid content between a blanched freeze-dried carrot and an unblanched freeze-dried carrot was statistically significant ($p = 0.005$). The impact from thermal processing on carotenoid content was observed by Lee et al. [14] and Rahat et al. [16]. Among others, Camorani et al. [17] noted a positive impact from thermal processing on the carotenoids content, which they attributed to the fact that at a high temperature cell walls were torn; consequently, the colour extraction was more efficient [18]. The increase in carotenoid content as a result of thermal processing was also confirmed by Azizah et al. [19]; Pellegrini et al. [20]; Pinheiro et al. [21]; Seybold et al. [22]. When analysing the changes in carotenoid content in processed tomatoes, they found the increase in carotenoids content. Cooking results in the degradation of protein, the softening of the cell walls and the release of carotenoids from these complexes, which facilitates the extraction of carotenoids. One more aspect seems worth noting. During the process of blanching, the air from intercellular spaces is removed and native enzymes are destroyed, which may reduce the oxidation process.

During the storage of freeze-dried carrots in atmospheric air, after 2 weeks a 10 % decrease was noted in carotenoids content, both in blanched and unblanched carrot slices. After 4 weeks of storage, carotenoid content fell by 30–50 %, while after 16 weeks there was only about 5 % of the initial content of carotenoid in carrot (Figure 1). These results show the intensity of the carotenoid oxidation in the atmospheric air. Singh et al. [23] examined a decrease in carotenoid content in carrot powder and carrot grits being stored in different packaging (in light and without light) for 6 months. They observed a constant fall in carotenoid content, with 60–76 % after 6 months in the material immediately after drying, and 78–86 % in fresh carrot.

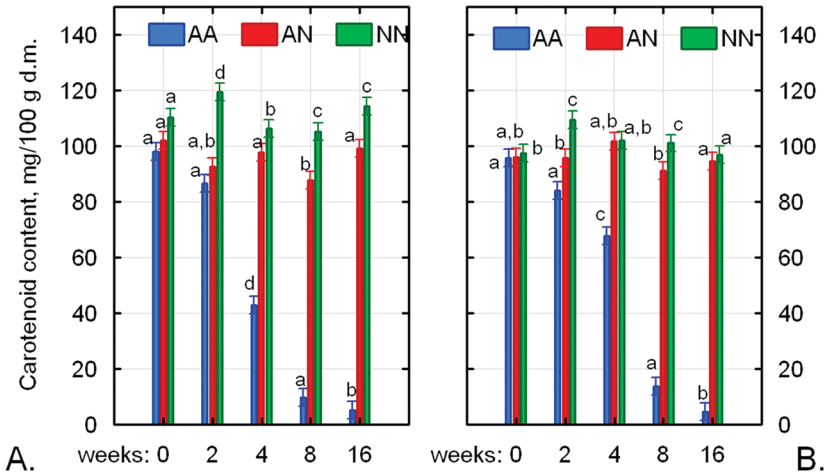

Figure 1: Effect of pre-treatment, atmosphere and storage period on changes in carotenoid content: (A) blanched material; (B) unblanched material; AA – air-filled carrot slices packed under air atmosphere, AN – air-filled carrot slices packed under nitrogen atmosphere, NN – nitrogen-filled carrot slices packed under nitrogen atmosphere. Results are expressed as mean of four replications. Vertical bars represent confidence interval 0.95. Different letters (a–d) mean significant difference ($p < 0.05$) at the same time.

When the carrot structure was filled with nitrogen, whether by filling a drying chamber with nitrogen or by packing in an nitrogen atmosphere, the carotenoids content did not change in a statistically significant manner during the whole storage period (Figure 1). An increased carotenoid content in the material marked as BL_NN (Table 1) noted immediately after drying remained stable after the storage period.

3.2 The colour

The colour expressed as total colour difference ΔE, after storage, are shown in Figure 2. The colour was measured both on the surface and inside the slices, in cross sections, perpendicularly to the axis of the slices. The aim of this technique was to verify the hypothesis that due to high porosity the colour changes caused mainly by oxidation of carotenoids or the crystallization of dry substance components, took place simultaneously in the entire volume of the material tested.

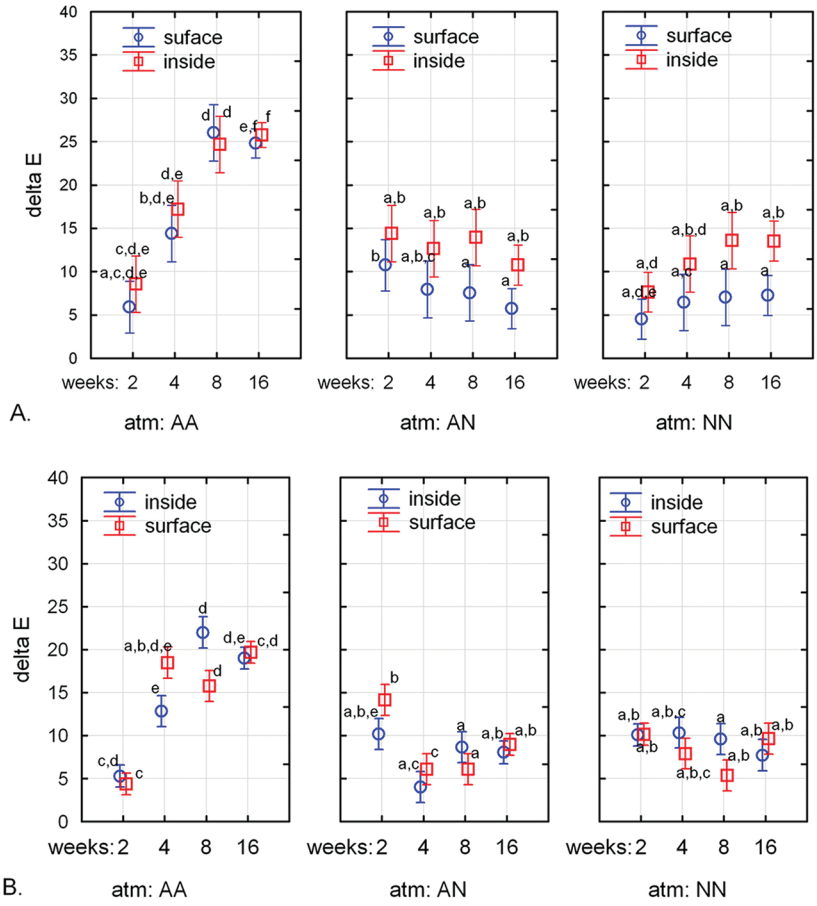

Figure 2: Effect of atmosphere, storage period and colour measurement on changes in colour: (A) blanched material; (B) unblanched material; AA – air-filled carrot slices packed under air atmosphere, AN – air-filled carrot slices packed under nitrogen atmosphere, NN – nitrogen-filled carrot slices packed under nitrogen atmosphere. Results are expressed as mean of five replications. Vertical bars represent confidence interval 0.95. Different letters (a–f) mean significant difference ($p < 0.05$) at the same time.

The ΔE difference was determined with reference to the colour determined immediately after drying (time 0). This enabled the impact of storage period and the elimination of the impact of water content on the colour (only on the dry material) to be determined. The most significant changes in colour were noted in carrot slices packed in the atmospheric air, irrespective of pre-treatment. After 2 weeks of storage ΔE was 4.38–8.55. After 8 weeks it was 24.69–26.02 for blanched carrots (Figure 2A) and 18.76–22.00 for unblanched carrots (Figure 2B). These values did not change in a statistically significant manner after another 8 weeks of storage. Lee et al. [14] noticed a change in the ΔE parameter as a result of thermal processing, amounting to 4.51–24.71.

For the carrot slices filled with air (AN) or nitrogen (NN) after freeze-drying and storage in nitrogen atmosphere (AN and NN materials, Table 1) no change in the ΔE parameter was noted during storage, which confirms the stability of the colour. This correlates with the carotenoid content results obtained. Since the main ingredients of carrots, influencing the colour, are carotenoids [24], the unchangeability of their content should result in the unchangeability of colour. This correlation was confirmed. Neither was there a difference noted in the ΔE parameter for the AN and NN materials although the carotenoid content was noticeable in BL_NN material (Table 1). This could be due to the fact that carrot are a non-homogeneous material, of various colouring depending on where it is measured. This is confirmed by the high standard deviation value of up to 40 % of the determined value. Therefore, colour measurement is not very sensitive to slight changes in carotenoid content. It is worth stating that no statistically significant impact from the test results on the colour changes between the surface and the inside of the material was found. It means that the colour changes, if any, were observed in their entire volume of freeze-dried slices (material BL_AA and NB_AA). Due to the high porosity of the material, it is easy for the surrounding air to migrate into the inside of the structure. Carotenoids undergo oxidation, and this causes the change in colour.

3.3 Sorption properties

The changes in the relative water content during sorption in the materials immediately after drying and after storage are presented using the example of the BL_NN material in Figure 3.

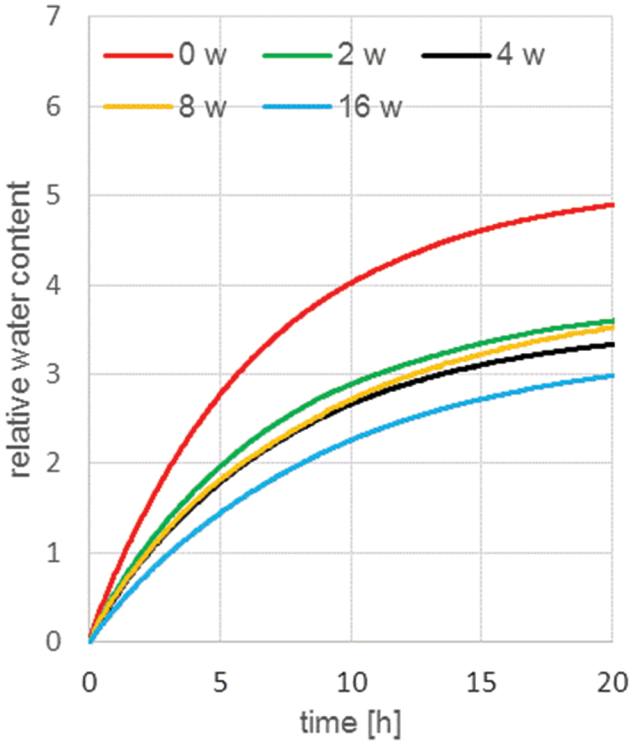

Figure 3: The changes in sorption properties during storage: w – weeks, time of storage.

Sorption was the most intense during the first 10 h of the process. In this period, about 75–80 % of water was adsorbed. The highest sorption ability was noted in the material immediately after drying. During storage, the sorption ability decreased. After 16 weeks of storage the material absorbed about 35 up to 40 % less water than immediately after drying. A statistical analysis was carried out assessing the significance of the differences in relative water content after 20 h of sorption in order to determine the statistical significance of material variation in terms of sorption ability. The results obtained are presented in Figure 4. The most statistically significant changes in water sorption ability were observed during the first 2 weeks of storage for each of the materials tested. These changes indicate the transformation of dry matter components from the amorphous form, characterised by a high sorption ability, to a crystalline form. The changes in sorption properties during 2 weeks of storage of freeze-dried apples were observed by Nowak [25]. The pictures of the microstructure taken with the use of an electron microscope led to the observation of the crystalline structures created during storage.

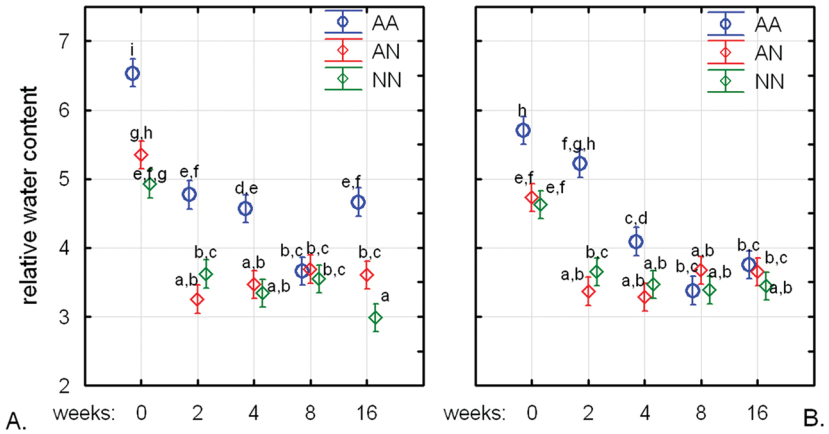

Figure 4: Sorption properties – a relative increase in the water content after 20 h of sorption: (A) blanched material; (B) unblanched material; AA – air-filled carrot slices packed under air atmosphere, AN – air-filled carrot slices packed under nitrogen atmosphere, NN – nitrogen-filled carrot slices packed under nitrogen atmosphere. Results are expressed as mean of three replications. Vertical bars represent confidence interval 0.95. Different letters (a–i) mean significant difference ($p < 0.05$).

Moreover, the changes in the colour may result from the physical state of the ingredients.

4 Conclusion

In order to preserve high nutritional value of freeze-dried carrot slices, they should be stored in a nitrogen-enriched atmosphere. In the atmospheric air, even with no light, after a short time (16 weeks) the content of carotenoids in freeze-dried carrot slices was reduced to approximately 5 % of initial values. These changes are noticeable due to a significant change in colour. The infusion of carrot slices with nitrogen allows the content of carotenoids to remain unchanged over the storage period. When the freeze-dryer chamber was filled with nitrogen after the process, the freeze-dried carrot structure was additionally protected against oxidation. Filling the freeze-dryer chamber with air promoted oxidation. Despite packing in nitrogen, the content of carotenoids in the dried carrot decreased by about 5 %.

Blanching carrot slices before drying led to the retention of carotenoids, probably due to the deactivation of enzymes, especially peroxidase.

In all dry materials tested during storage, a decrease in the water sorption ability was observed. The absorption capacity of water by freeze-dried carrots decreased during 16 weeks of storage by nearly 40 %, with a decrease of 30 % after the first 2 weeks of storage. This indicates large physical changes in the material shortly after freeze-drying. These changes may have been due to the transformation of the dry matter components from the amorphous state to the crystalline state. It also proves that the material needs to be protected immediately after freeze-drying

It seems that blanching and infusing the porous structure with nitrogen while reducing the vacuum in the drying chamber provides additional protection which prevents carotenoids loss during storage.

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